Anti-Phospho-MEK1/2 (S218 + S222) Antibody [ST0490]

ET1609-50

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 43 kDa
Clone number:	ST0490

Description: Activation of extracellular signal-regulated kinase (ERK) or mitogen-activated protein kinase by MEK (mitogen-activated protein kinase or extracellular signal-regulated kinase kinase) is an essential event in the mitogenic growth factor-induced signal transduction pathway. Phosphorylation of MEKs correlates with their ability to phosphorylate and activate ERKs. MEK1 and MEK2 can also be activated by autophosphorylation. Lipopolysaccharide activates many of the MAPK family members of the immediate upstream MAPK activator MEK1, MEK2, and MEK3. In plants, MEK can phosphorylate and activate MAPK, and that Tyr phosphorylation is critical for the catalytic activity of MAPK in plants.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser218 and 222 of Human MEK1 aa 200-249 / 393.

Positive control: NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate, C6 treated with 200nM PMA for 30 minutes cell lysate, Hela cell lysate, A431 cell lysate, 293T cell lysate, NIH/3T3 cells treated with 200nM PMA for 30 minutes, Hela, HepG2, human tonsil tissue, human liver carcinoma tissue, human spleen tissue, human breast carcinoma tissue.

Subcellular location: Cytoplasm, Cytoskeleton, Membrane, Nucleus.

Database links: SwissProt: Q02750 Human | P31938 Mouse | Q01986 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

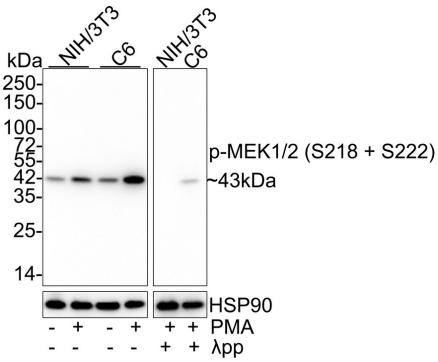

Fig1: Western blot analysis of Phospho-MEK1/2 (S218 + S222) on different lysates with Rabbit anti-Phospho-MEK1/2 (S218 + S222) antibody (ET1609-50) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 200nM PMA for 30 minutes cell lysate
Lane 5: NIH/3T3 treated with 200nM PMA for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour
Lane 6: C6 treated with 200nM PMA for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-50) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

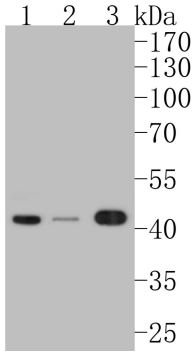

Fig2: Western blot analysis of Phospho-MEK1/2 (S218 + S222) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1609-50, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: A431 cell lysate
Lane 3: 293T cell lysate

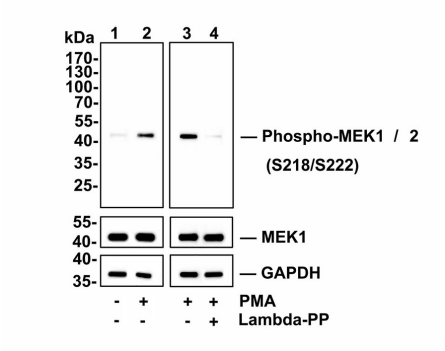

Fig3: Western blot analysis of Phospho-MEK1/2 (S218 + S222) on Hela cell lysates.

Lane 1: Hela cells, whole cell lysate, 10ug/lane
Lane 2/3: Hela cells treated with 200 nM PMA for 20 minutes, whole cell lysates, 10ug/lane
Lane 4: Hela cells treated with 200 nM PMA for 20 minutes, then treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-MEK1/2 (S218 + S222) antibody (ET1609-50) at 1/500 dilution. Anti-MEK1 antibody (ET1603-20) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size:43 kDa
Observed band size:43 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: Lane1/2 5 minutes
Lane3/4 1 minutes 32 seconds

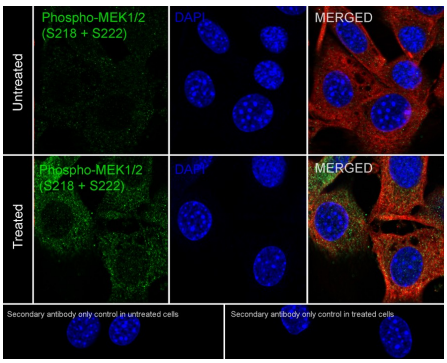

Fig4: Immunocytochemistry analysis of NIH/3T3 cells treated with 200nM PMA for 30 minutes labeling Phospho-MEK1/2 (S218 + S222) with Rabbit anti-Phospho-MEK1/2 (S218 + S222) antibody (ET1609-50) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-MEK1/2 (S218 + S222) antibody (ET1609-50) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

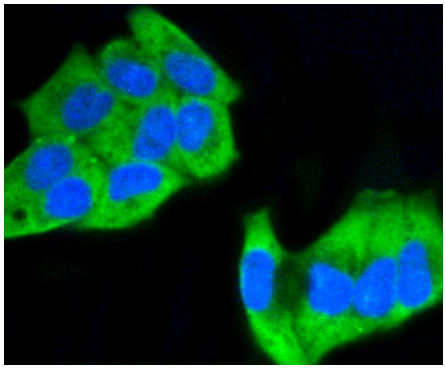

Fig5: ICC staining of Phospho-MEK1/2 (S218 + S222) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-50, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

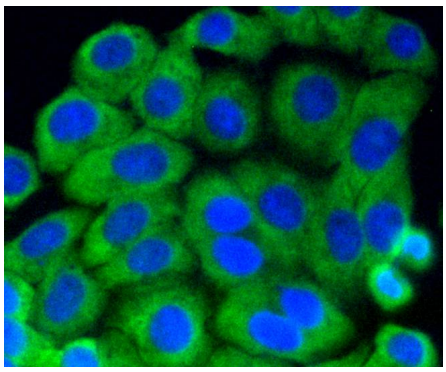

Fig6: ICC staining of Phospho-MEK1/2 (S218 + S222) in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-50, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

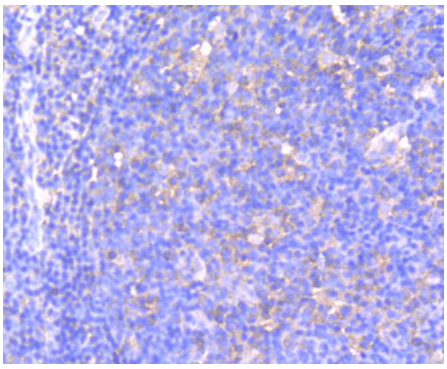

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Phospho-MEK1/2 (S218 + S222) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-50, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

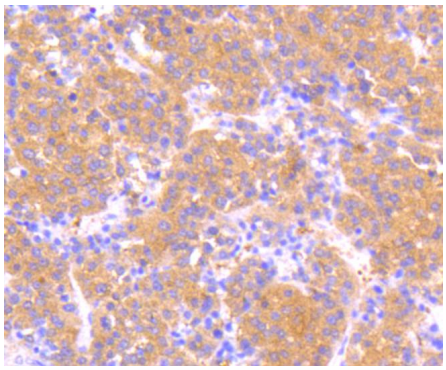

Fig8: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-Phospho-MEK1/2 (S218 + S222) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-50, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

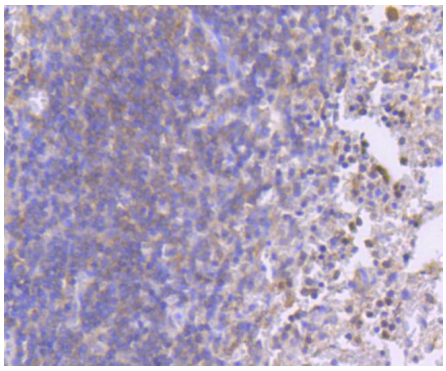

Fig9: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-Phospho-MEK1/2 (S218 + S222) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-50, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

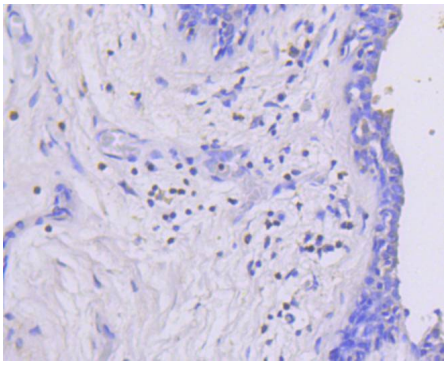

Fig10: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-MEK1/2 (S218 + S222) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-50, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Wortzel I., Seger R. The ERK cascade: distinct functions within various subcellular organelles. Genes Cancer 2:195-209(2011).
2. Bian Y., Song C., Cheng K., et al. An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. J. Proteomics 96:253-262(2014).